REMARKS

By the subject amendment, Applicant has cancelled Claim 10 without prejudice and amended Claims 8-9 to better define the invention, as fully supported by an enabling disclosure. More specifically, Claim 8 is recast as a method for selectively alkylating β-tubulin in cells. Claim 9, dependent on claim 8, is specifically directed to alkylating β-tubulin in human breast carcinoma cells.

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

Rejection under 35 USC §102 and 35 USC §103

Claims 8-10 remain objected to by the Examiner under 35 USC 102(b) as being anticipated by or, in the alternative, under 35 USC 103(a) as being obvious over Poyet *et al.* (CA 121: 339, 1993).

The Examiner alleges that Poyet *et al.* teach that tBCEU (N-(2-chloroethyl)-N'-[4-(1,1-dimethylethyl(phenyl]-urea) "inhibits the formation of tubulin and vimentin in human breast cancer cell line as an antitumor mechanism" and that "the compound taught by *Poyet et al.*, alters the synthesis of tubulin and vimentin and by that route treats human breast carcinoma". The Examiner is of the opinion that former claims 8-10 are drawn to the same method of treatment. Therefore, the Examiner concludes by alleging that disrupting tubulin would inherently disrupt β-tubulin and thus would make obvious the present invention.

The Applicant respectfully submits that although the Examiner points out that the compound taught by Poyet *et al.*, "alters the synthesis of tubulin and vimentin

and by that route treats human carcinoma", it does not anticipate or render obvious the identification of the compounds of the present invention as β-tubulin alkylating agents for the following reasons.

Firstly, the Applicant would like to clarify the following statement made by the Examiner:

> "the compound taught by Poyet et al., (tBCEU) inhibits the formation of tubulin and vimentin in human breast cancer cell line"[Emphasis added].

It is respectfully submitted that the experimental facts are much more nuanced. The Examiner is particularly referred to Poyet et al., at page 1448, second column, second paragraph. This section entitled "Effect of tBCEU on tubulin and vimentin synthesis" states that:

> "tBCEU **stimulated** the synthesis of tubulins when present at concentrations ranging from 5 –to- 13 μM. At higher concentrations, however, tBCEU decreased their synthesis." "...at the lowest concentration (2μM) of tBCEU used, we observed significant stimulation in the synthesis of vimentin, an effect which become maximal at 50 μM." [Emphasis added]

The Examiner is also referred to Poyet *et al.*, at page 1449, second column 2, lines 1and 2.

> "The results clearly indicate that tBCEU **increases the synthesis** of at least 2 proteins: **α-tubulin** and vimentin"[Emphasis added].

Thus, overall, Poyet *et al.*, show an increase of α-tubulin and vimentin synthesis, not an inhibition of the formation of tubulin and vimentin. Poyet et al. merely report an alteration in the synthesis pathway of tubulin, more particularly of α-tubulin. These results neither demonstrate nor suggest that tBCEU specifically alkylates β-tubulin.

Poyet *et al.*, observed, in addition to the increase in the formation of α-tubulin and vimentin proteins, that tBCEU simultaneously decreases the accumulation of tubulin (α and β) and vimentin mRNAs. Poyet *et al.* suggested that the antineoplastic activity of tBCEU is in part related to an alteration in the synthesis pathway of tubulin and vimentin. Poyet *et al.*, hypothesized that this alteration could be due to the following different mechanisms (see the discussion on possible mechanism of action beginning at page 1450, first column):

1) Direct action on the synthesis pathway by triggering and/or suppressing a factor involved in this pathway.

2) Indirect action on the synthesis pathway by a direct action on the microtubules (see page 1450) via:

a) a direct binding of the drug to the microtubule; or

b) the alteration of some posttranlational process on tubulin.

Of course, a possible direct action of tBCEU on tubulin is discussed but this is only one possibility out of several other mechanisms of action that are discussed. Therefore, Poyet *et al.* only speculate that the action of tBCEU might be initiated by its activity on microtubule assembly. No results demonstrating the actual direct action of tBCEU on microtubule or tubulin, are presented by Poyet *et al.*

Furthermore, the notion of tubulin disruption is discussed at p. 1450 in the context of hypothesis 2a (direct binding of the drug to microtubules). It is suggested that tBCEU could act via pathways that could be similar to the general class of antimicrotubule agents. Different mechanisms of action direct the activity of anti-microtubule agents. Some of these agents do react with free tubulin, others with tubulins assembled into microtubules. However, none of them alkylate tubulin. This alkylating property is a unique characteristic of tBCEU that was demonstrated in work subsequent to Poyet *et al.*

The Examiner is particularly referred to page 1450, first column where Poyet et al., discusses the different hypotheses concerning the effect of tBCEU on the tubulin synthesis pathway:

> "**We do not know, however, whether the action of tBCEU on tubulin synthesis pathway was direct or indirect**. As tBCEU is cytotoxic for the MDA-MB-231 cells, it **could** trigger and/or repress the synthesis of a factor that can then alter at the end the tubulin synthesis pathway. **The effect of tBCEU on tubulin synthesis might also be mediated by a direct** action on microtubules. It **may** disrupt the microtubule pathways that are similar to other antimitotic agents: colchicin (19), vinca alkaloids (20), podopyllotoxins derivatives (21) and taxol (22). **A close look at the activity of these drugs shows that they have different effects on tubulin synthesis and it is possible that tBCEU acts through one of these mechanisms.**" [Emphasis added]

Thus, in conclusion, Poyet *et al.*, only speculate on the mechanism of action of tBCEU. Poyet *et al.*, only demonstrate an effect of tBCEU on tubulin synthesis. However, Poyet *et al.* do not demonstrate a direct effect on tubulin and even less so on β-tubulin. The Applicant therefore respectfully submits that Poyet *et al.* does not anticipate nor render obvious the instant claims.

In view of the above, the Applicant respectfully requests favorable reconsideration of the present application. The present application is believed to be in condition for allowance and a notification to this effect is earnestly solicited.

This response is being submitted along with a request for a two month extension of time to respond and a check in the amount of $215.00. It is believed that no additional fee is due for this submission. Should that determination be incorrect, however, the Examiner is hereby authorized to charge any deficiencies to our Deposit Account No. 50-0562, and notify the undersigned in due course.

Respectfully submitted,

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